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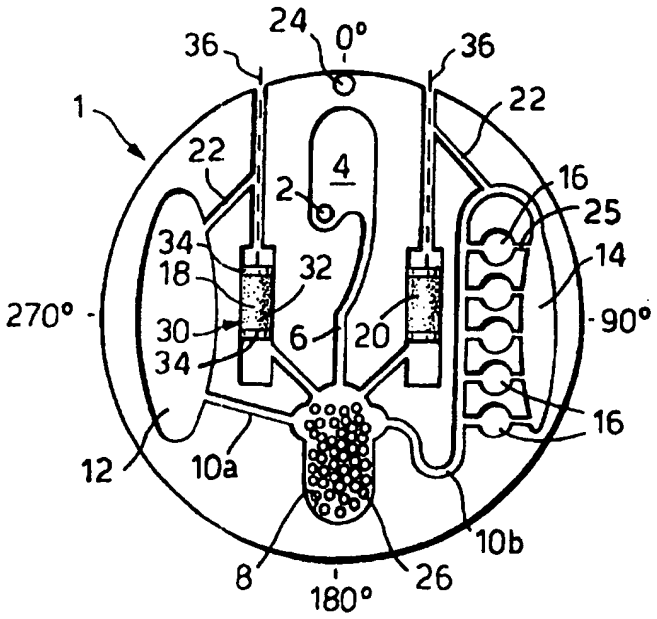
(57) Abstract

The invention relates to an apparatus and method for chemical analysis. In a preferred embodiment the invention comprises a disc shaped member (1) which has a chamber (8), pathways (10a and b), reservoir (12) and analysis cells (16) formed integrally therewithin. A blood sample is introduced into the apparatus and is centrifuged so as to separate blood cells from serum. Serum is then subjected to a number of processes and/or tests, for example, in order to establish the state of the serum. The invention overcomes problems of prior art systems by being able to repeat certain processes, for example cyclical tests or reactions, by causing or permitting a sample to flow into a region or volume then allowing it to flow out, then allowing it to flow into the region or volume again. Thus washing or rinsing is possible. Optionally sealed pistons (32) which are housed in the disc (1) contain liquid reagents or solvents or water. A matrix of beads (26) whose surface has been coated with a reagent may be disposed in a reaction chamber.



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Apparatus for and Method of Chemical Analysis

The present invention relates to an apparatus for, and a method of, chemical analysis, and more particularly, but not exclusively, to bio-chemical analysis.

A growing trend in chemical analysis is the development of assay methods and analysis means which are intended for "one-shot" use. In such devices the analysis means is disposed of after the analysis has been performed, together with the biological sample itself. This is convenient from an operator's point of view, in that there is no equipment to maintain and calibrate, and it is especially important in respect of hygiene. A further advantage is the elimination of cross-contamination between sequential samples, an important issue in DNA testing.

A common feature of wet chemistry analysis systems is that an analyte, together with one or more reagents, passes sequentially through a series of various procedures, such as mixing, filtering, decanting, metering and dividing. More advanced assay methods, such as polymerase chain reaction (PCR) amplification of DNA, require samples to be thermally cycled a number of times, and it is common to use optical means for detecting end products of the reaction or assay.

In short, some form of fluid-handling means is a common and important feature of most assay systems, even when the assays themselves might be different from each other in scope and purpose.

US Patent No. US-A-5,160,702 (Molecular Devices Corp) describes an analyser wherein a sample for analysis is introduced into a small cell in a synthetic plastics disc. The sample is then moved through a series of adjacent cells by a varying combination of capillary action and centrifugal force, the latter created by spinning the disc about its centre point. Adjacent cells are configured to perform various functions such as separating out solids and differing density materials from the analyte solution.

In practice, however, there are several drawbacks when using capillaries formed in moulded plastic devices. Some of these are listed below:

1. Capillaries are difficult to mould accurately in synthetic plastics.
2. Narrow channels are very susceptible to blockage.
3. The wettability of synthetic plastics surfaces (and hence capillary properties) may vary.
4. Very large pressure gradients are needed to reverse the capillary flow.
5. Multi-part capillary cell assemblies are difficult to join together without creating lateral leakage as a result of capillary action in pathways.
6. Visual observation of any reaction process is difficult because the disc is spinning.
7. There are limitations in the storage and introduction of reagent fluids, because the use of centrifugal forces to release fluids is not selective. For example, if two or more rupturable seals, (which seal volumes of reagent), are activated at a certain g-force, were deployed, then all the seals would be ruptured at the same time. This is because it is not practical to arrange seals which are triggered at different g-forces.

Also, the use of centrifugal action to provide a variable g-force has drawbacks, in that the control of electrical power to the motor is difficult and expensive, and requires a relatively large, bulky electric motor.

US Patent No.US-A-5,162,237 (Miles Inc) discloses a rectangular cassette, into which a blood sample is introduced for mixing with one or more reagents. Mixing is followed by a visual inspection or optical measurement. The cassette is concerned with efficiently mixing the various fluids by oscillating an assembly containing fluid-obstructers back and forth, so that the fluids are intermixed.

It is not possible to carry out a plurality of separate processing stages in a controlled sequence using any of the prior art systems.

An object of the present invention is to provide a compact apparatus and method for carrying out wet chemistry assays having a plurality of processing stages, which apparatus and method can be configured for a wide variety of assay types. Additionally,

the apparatus is desirably low-cost, easy to operate and suitable for use away from the laboratory bench.

According to the present invention there is provided apparatus for chemical analysis comprising: a member having formed therein a reaction chamber, which is in fluid communication with at least one analysis cell, at least one reservoir and at least one centrifuge chamber, the at least one centrifuge chamber is adapted to receive a sample via an inlet port, so that, in use, a sample passes from the centrifuge chamber into the reaction chamber when the member is placed in a first orientation; the sample flows from the reaction chamber into a first reservoir when the member is placed in a second orientation; and the sample flows from a reservoir to the reaction chamber when the member is placed in a third orientation; and the sample flows from the reaction cell to at least one analysis cell when the member is placed in a fourth orientation.

Preferably the first and third orientations are substantially identical, thus the sample is caused or permitted to return to a previous chamber.

In a particularly preferred embodiment the member is rotatable in opposite senses at an angular velocity which is less than the angular velocity of a centrifuge which supports the member. Thus by repeated cycling of the member from one orientation to another, it is possible to achieve, for example, washing or rinsing of the sample. Advantageously displacement of the member from one orientation to another is achieved by stepping the member through predetermined paths, such as, for example arcs of circles.

The aforementioned apparatus may be used to analyse chemicals and/or in diagnosing diseases.

The reservoirs, chambers and pathways may be defined so that they lie at different heights from a base level on the member. Interconnecting fluid pathways or conduits may be sloped or all features may be formed so that they lie on one level.

According to another aspect of the invention there is provided apparatus for chemical analysis comprising: a rotatable member having formed therein a sample inlet port

communicating with a centrifuge chamber, a reaction chamber communicating with the centrifuge chamber by a fluid pathway, and at least one analysis cell communicating with the reaction chamber by a fluid pathway, wherein the rotatable member is spun, in use, in order to centrifuge a sample to be analysed within the centrifuge chamber, and wherein the rotatable member may subsequently be stepped for rotation at least partly in a vertical plane through fixed and predetermined arcs of circles so that the sample for analysis flows into the reaction chamber under the influence of gravity for reaction with one or more reagents, and the products of the reaction chamber subsequently flow into the at least one analysis cell.

In accordance with a further aspect of the invention, there is provided a method of chemical analysis comprising: providing a rotatable member mounted for rotation having at least a component in a vertical plane, the rotatable member having formed therein a centrifuge chamber, a reaction chamber communicating with the centrifuge chamber by a fluid pathway, and at least one analysis cell communicating with the reaction chamber by a fluid pathway,

introducing a sample for analysis into the centrifuge chamber and spinning the rotatable member to effect a centrifuge operation on the sample;

stopping the member spinning and rotating the member through a first predetermined arc of circle so that centrifuge products are caused or permitted to flow, via a fluid pathway, under the influence of gravity to the reaction chamber,

treating the centrifuge products in the reaction chamber with one or more reagents, and

rotating the member through a second predetermined arc of circle in order to cause or permit the contents of the reaction chamber to flow under the influence of gravity into the at least one analysis cell.

Preferably the member experiences a plurality of steps of rotation and counter-rotation so as to cause or permit a sample in the reaction chamber to experience a repeated set of reactions and/or processes. An example of this may be where a sample has bound chemically to a substrate, for example supported on a surface of beads, and repeated steps of washing or rinsing of the beads is effected.

Thus using the present invention, a controlled sequence of processing stages can be performed. Samples may be moved under the influence of gravity, through fluid pathways which are of sufficiently large diameter that capillary forces do not obstruct fluid flow. Generally the pathways are approximately 1 mm in diameter. More specifically the pathways are greater than 0.5 mm diameter.

The rotatable member is preferably in the form of a flat disc, formed from a synthetic plastics material having at least one surface which is substantially transparent to radiation in the visible waveband. The transparent surface permits visual inspection and facilitates monitoring of progress of reactions. Most preferably the member is disposable after a single analysis so as to avoid contamination.

In a preferred embodiment of the invention the reaction chamber contains an aggregate of small glass or ceramic beads, which have been coated with a reagent, for example an antibody, or onto which a reagent may be applied during operation. This use of these glass or ceramic beads (hereinafter referred to as microbeads) induces natural turbulence when fluids are passed into the reaction chamber and percolate through the matrix defined by the beads. Also the beads expose a large surface area of reagent to the sample fluid. As beads are used, there is a relatively short diffusion pathway from any point in the fluid to the nearest bead surface In practice the inter-bead separation is of the order of tens of microns.

According to a further aspect, the invention provides apparatus for chemical analysis comprising a rotatable member having formed therein a reaction chamber including an aggregate of beads for receiving on their surface a reagent, and at least one analysis cell communicating with the reaction chamber by a fluid pathway, wherein the rotatable member can be stepped for rotation through fixed and predetermined arcs of circle so that the products of the reaction chamber subsequently flow into the at least one analysis cell.

In a preferred embodiment liquid reagents are introduced into the reaction chamber by providing in the rotatable member one or more compressible, open-cell, structures, such as sponges, in which a liquid reagent is stored. A piston or other means of receiving and storing liquids may be provided for compressing the open-cell member in order to

squeeze therefrom a liquid (which may be a reagent or a rinsing medium) into a fluid pathway communicating with the reaction chamber. Several such pistons may be provided. In a particularly preferred embodiment first and second open-cell members are provided in separate cylinders, each containing a different liquid reagent from the other, and both being independently operable by first and second pistons.

According to a yet further aspect of the present invention there is provided apparatus for chemical analysis comprising: a rotatable member having formed therein a reaction chamber, at least one analysis cell communicating with the reaction chamber by a fluid pathway and storage means for introducing into the reaction chamber one or more liquids for acting on a sample, the storage means comprising a compressible, open-cell member adapted to be mounted within a chamber, the storage means, in use, contains a liquid, so that on compression the liquid is released into a fluid flow path communicating with the reaction chamber.

The invention extends to an adaptor for connecting the member to a centrifuge, the invention also extends to a centrifuge system which is adapted to receive the aforementioned disc member and is capable of being controlled so as to be able to operate selectively in a high speed centrifuge mode and also a relatively low speed stepping mode, thereby enabling relatively slow clockwise and slow counter clockwise rotation of the member.

Preferably the centrifuge system has a stepper motor which may be directly linked to a main, centrifuge rotor or may be connected thereto by way of a gear system.

Control of the system may be manual or by way of a computer.

A preferred embodiment of the invention will now be described, by way of example only, and with reference to the Figures, in which:-

Figure 1 is a plan view of a rotatable disc member;

Figure 2 shows details of a compressible member for receiving a fluid, together with illustrative views of the principle of operation thereof;

Figures 3 and 4 show different views of the method of operation of the apparatus, employing the embodiment shown in Figure 1; and

Figure 5 is a sectional, exploded view showing the disc member, a cap member and a rotor mounting of a centrifuge.

Referring now to the Figures in which Figure 1 shows the apparatus in the form of a disc member 1. The disc member 1 includes an inlet port 2 communicating with a centrifuge chamber 4, coupled by a fluid pathway 6 to a reaction chamber 8. The reaction chamber 8 is coupled by various fluid pathways 10 to different reservoirs which include: a waste depot chamber 12, an array of analysis cells 16, and first and second liquid injection chambers 18, 20. Breather tubes 22 are also provided. Index mark 24 is a start position from which the sense of rotation of the disc 1 is defined at various angular positions. The purpose of this is explained below.

Each analysis cell 16 (fixed-volume metering cell) contains a particular enzyme which reacts with an antigen present in the test sample, causing either a colour change in a solution which supports a sample, or inhibition of induced fluorescence of the solution. Various methods of colorimetric analysis are known, both for visual inspection, and for automated detection and measurement. Pathway 10b to the analysis cells 16 is wide-bore, but outlet bores 25 to the waste depot 14 are narrow-bore, for reasons explained below.

Disc 1 subjects an introduced sample (the analyte) to one or more wet-chemistry processing stages, involving wet reagents, dry reagents, or both, so that the sample is processed using similar methods and materials as would be present in a conventional laboratory. This is achieved entirely within the apparatus. Thus the apparatus confers several major benefits, including flexibility of operation, hygienic operation, freedom from cross-contamination between samples, and long shelf-life.

Typically, the apparatus is in the form of a 70 mm diameter disc, 5 mm thick, although other shapes are equally viable (such as squares or hexagons). Fluid pathways 10a and 10b are greater than capillary size, for example 0.2 mm, and typically 0.5 mm diameter, so that fluids can flow relatively freely under natural gravitational action. Injection chambers for storing liquids and dispensing liquids are, (described in detail below with reference to Figure 2), are 3 mm diameter and 10 to 20 mm in length. The dimensions are consistent with processing sample sizes in the range approximately 30 to 500 microlitres.

In use reaction chamber 8 includes an aggregate of glass beads 26 whose diameter is approximately 0.6 mm. This diameter ensures the beads are large enough not to be flushed from the chamber in which they reside, but small enough to provide a reasonably large surface area, accessible easily by diffusion processes within the sample fluid. Ideally all locations in the fluid are within 100 microns of the nearest bead surface. Beads 26 are a convenient vehicle to immobilise chemicals on, because they can be "poured" into place, and also machine-counted. The extreme practical range for bead diameter is 0.1 mm to 3.0 mm. Preferably the diameter of the beads is in the range 0.5 to 1.5 mm.

Injection chambers 18 and, 20 each contain a cylindrical reservoir 30 which acts as an injector device. Inside each chamber there is located a foam material 32 having impervious upper and lower faces 34. An absorbent foam plastic, sponge-like material is preferred for the piston body 32: Such material is defined as having an open-pore structure. This means the material absorbs and retains fluids. The upper and lower faces 34 of the piston 32 can also be formed from foam plastic. The upper and lower faces 34 of the piston 32 are impervious so as to prevent evaporation or leakage of any fluid absorbed when the piston body is charged. Alternatively, upper and lower faces comprise silicone rubber materials, or wax/grease impregnated materials. These materials also ensure a good conformal fit to the inside surfaces of the apparatus which define the cylinder(s).

Pistons 32 of this type are made by coating a sheet of absorbent foam material (having a thickness equal to the length of the required pistons) with the impervious materials on the

upper and lower surfaces, and then punching out the cylinders with a rotating, hollow drill-type system (not shown). Actuator rods 36, indicated in dotted lines, are optionally provided for compressing pistons 32. Alternative actuators means may be provided.

Referring to Figure 5, which is a sectional view of disc 1 and a rotor mounting, it may be seen that the disc 1 has a transparent plate 50 bonded to the body of the disc 1. The lower surface of the disc 1 has a central hemispherical alignment guide 52 and an alignment dog 54 disposed in it's periphery. Guide 52 and dog 54 mate with corresponding guides 62, 64 of disc mount adapter plate 60, adapted to be mounted onto a main rotor element 70 of an electric motor (not shown) by bolts 72 and bolt holes 74. Adapter plate 60 has two diametrically opposed flexible members 66. In use, members 66 can be moved outwardly by finger pressure to allow the disc to be engaged in position in the adapter plate 60, and then allowed to snap back over the disc to retain the disc in position. The rotor is mounted at an angle of 45 degrees to the vertical, so that the disc is also at a similar angle.

Figures 2a to d, illustrate diagrammatically the concept of operation of the reservoir 30 and piston 32. A cell is shown containing a foam piston 32, as described above, and a chamber "A", into which reagent is to be injected at a particular time. During manufacture, the piston 32 is partially inserted into the cylinder 30, leaving a short section of the body of the foam piston 32 exposed. Just before packaging, a fixed, metered quantity of liquid reagent is added via an automated titrator (not shown) to the exposed foam piston 32. The foam absorbs the reagent, and the piston 32 is then pushed into the cylinder 30, so that the upper, impervious face 34 is flush with (or slightly below) the cell surface, as shown in Figure 2a. In this condition, there is no evaporative pathway for any fluids to leave the reservoir. It is hermetically sealed by the cylinder walls and by the impervious faces 34 on the piston 32. When required to transfer reagent into chamber "A", a pushrod (actuator 36) is inserted in the direction of the arrow so as to compress the piston, as shown in Figure 2b. Because the piston 32 is hermetically sealed, it moves downwards in the cylinder until the lower impervious face moves below connector tube 33, shown in Figure 2c. At this stage, there is a fluid pathway along tube 33 for the reagent to escape from the foam piston 32 into chamber "A", and so further

compression of the piston 32 injects the reagent into chamber "A". When the piston has been entirely compressed, the pushrod is withdrawn, and then either the piston remains collapsed, or springs back, absorbing air via breather tube 35 and upper region of chamber "A". This is dependent on materials and cell configuration. In either event, a precise amount of reagent has been injected into the requisite compartment at the chosen time. The injection process can be either fast or slow, depending on requirements, and it can also be carried out in stages (e.g. four, 25% titrations).

Referring now to Figures 1, 3 and 4, which show a typical sequence of events for an immunological assay using the present invention, the positional changes of the disc 1 can be followed by observation of index mark 24 adjacent centrifuge chamber 4. After mounting the disc to a centrifuge machine using the adaptor shown in Figure 5, the sequence of events is described below.

The sequence described comprises eight steps. It will be appreciated that many other different sequences are possible

1. Sample acquisition. A blood sample (30 µl approx.) is injected into centrifuge chamber 4 via the inlet port 2, with disc 1 set to position 180°. In this position, the axis of centrifuge chamber 4 is vertically downwards and liquids tend to remain in the centrifuge chamber.

2. Red-cell separation. The blood sample is "spun-down" by the centrifuge to precipitate red blood cells in the sample. The disc 1 is then restored to position where marker 24 is adjacent 180°, i.e., with the centrifuge chamber 4. The position is shown in Figure 3b.

3. Serum separation and affinity trapping. Blood serum in the centrifuge chamber 4 is decanted into the reaction chamber 8 by stepwise rotation of the disc 1, in an anti-clockwise sense to position 0°, so that serum flows under gravity through pathway 6 into chamber 8, as seen in Figure 3c. Red cells are left behind in centrifuge chamber 4. During a short period, antigen in the serum bonds to an antibody which has been tagged (bonded) in a previous operation onto the surfaces of the glass (or ceramic) beads 26.

4. Serum removal. The serum is drained from chamber 8 along pathway 10 into reservoir 12 which is hereinafter referred to as primary waste depot 12. This occurs by rotating the disc to position 270° and is depicted in Figure 3d. Waste depot 12 contains a porous foam or filter-paper material (not shown) to trap entrained fluids. The antigen-antibody complexes in the serum remain tagged to the beads 26. The disc 1 is then restored clockwise to position 180°.

5. Washing. The disc is returned to the 0° position, (as described in STEP 3 above with reference to Figure 3C) and foam piston injector 30 is compressed using an actuator rod 36. This acts to inject a rinsing solution contained within the open cell foam piston 32, into reaction chamber 8. The washing stage is shown in Figure 4a. Movement of the disc to the appropriate angles as shown in the Figures is sufficient to move the solutions for mixing purposes. However, other types of agitation way be performed at this stage. If the disc 1 is spun slowly, upwards of 1 rev per second, i.e. fast enough to stop solutions leaving the reaction chamber, but not so fast that large g-forces are created, then the solution in the chamber around the beads is agitated, helping the reactions to occur more efficiently and faster. Alternatively a vibration bed (not shown) may be provided so as to achieve this.

6. Removal of wash solution. The rinsing solution is decanted into the primary waste depot 12 by rotating the disc to position 270° (shown in Figure 4b) and then restoring it anticlockwise to the 0° position. At this stage, only chemical entities in the reaction chamber 8 are the clean, antibody-antigen complexes.

7. Elution is performed in the 0° position, as shown in Figure 4c. An elute solution contained in piston 32 of chamber 20 is released into the reaction chamber 8 by compressing piston 32 and causing a change in the pH of the solution This disrupts antibody-antigen bonds, thus releasing the trapped antigen into solution again.

8. Metering and optical measurement. Finally, the reaction chamber contents are decanted into a plurality of fixed-volume analysis cells 16 for optical interrogation by

rotating disc 1 to position 90°. This is shown in Figure 4c. The fixed-volume analysis cells 16 comprise a relatively large-bore inlet port 10b leading to each fixed-volume cell. A relatively narrow-bore outlet tube leads from each cell to a common waste reservoir 14. Such a structure has a fluid impedance which is dependent on its level of fill. Fluid can flow relatively easily into each cell 16 until the narrow-bore section is reached, at which stage the force needed to continue driving the fluid into the cell rises greatly. At this point, the fluid, which flows most readily along the path of lowest resistance, effectively diverts to the conduit leading to an adjacent cell, and so on. Thus, each of the cells 16 fills efficiently, one by one. This sequential filling of cells ensures. Very little additional fluid flows in to each cell once it has been filled, and hence the effective volume residing in the cell is well-defined. This may be important from a qualitative measurement point of view.

Also by careful selection of the diameter of fluid pathways; variations in their cross-section; and the loops or bends formed by them, it is possible to introduce predetermined delay times for fluids flowing from one reservoir to another or from a reservoir to an analysis cell. Effectively the pathways can be formed so as to define a fluid logic circuit so that, for example, a sample may be divided into two or more volumes and one sub-sample may be directed along a pathway directly to a reaction chamber; whilst another sub-sample may be sent via a delay line to a different chamber in order that it is subjected to a different reaction at a different, predetermined instant.

Breather tubes 22 ensure that fluids can move freely by, expelling air ahead of themselves. Breather tubes 22 are configured so that they link non-critical volumes, ensuring any excess fluid does not interfere with the chemical analysis.

The disc-shaped embodiment of the invention may be conveniently manufactured in the form of two synthetic plastics mouldings, representing an upper-half and lower-half of the final assembly. The lower-half moulding contains indentations and recesses which define chambers, reservoirs, cells, conduit pathways and lower halves of injection cylinders. The upper-half moulding contains only indentation defining the upper halves of the injection cylinders. Additional elements can be located in the appropriate cavities

in either lower-half. The upper-half of the disc is bonded to the lower-half. Additional elements referred to in the non-exhaustive list, for;

1. Foam piston injectors (either pre-loaded with reagent or dry for subsequent loading prior to distribution).
2. Absorber material (for waste reservoirs or depots, such as porous paper pads).
3. Glass/ceramic microbeads, either passive or antibody-tagged.
4. Heparin-loaded gel or paper, for prevention of coagulation.
5. Enzyme-loaded paper discs (in read-out cells).

Bonding of the upper and lower halves may be achieved either using a porous-gasket technique described in the Applicant's granted US Patent No US-A-4,865,716, or modification thereof. For example, a gasket of tissue paper or polymer is impregnated with a wax or low-temperature thermal glue, and punched to shape, such that apertures are created in areas to be kept free from glue. Next, the gasket is sandwiched between upper- and lower-halves of disc 1, by snapping the two halves together. The assembly is then thermally cycled above the melt point of the wax (typically 55 °C) or glue (typically 110 °C), thereby sealing the arrangement. Mechanical registration features moulded into both halves of the disc assembly ensure very accurate alignment, and a "snap-fit".

It may thus be seen that the present invention, at least in the preferred embodiment, has the following advantages:

Modification to the aforementioned methods may be made so as to reflect embodiments.

1. All sample and reagent fluids are contained in a sealed environment, consequently the invention is hygienic in use.
2. The invention can be tailored to a wide variety of different assays, and is not specific to a single type.
3. The components are all low-cost, and manufacture and assembly is simple.
4. Automation of the system is practical, using simple control systems.

5. Visual check of the proceeding analysis is possible.

6. Hand-held (machine-free) versions of the invention are possible (for field-work and third-world use), wherein a credit-card sized version in the form of a square, pentagon or hexagon or the like can be stood on any of its edge(s), thus allowing manual rotation and positioning for the appropriate time periods and/or appropriate orders.

7. Internal fluid-dispensing means is selective: thus several different reagents can be administered at different times, and at different rates.

8. The internal fluid-dispensing means is accurate.

9. The internal fluid-dispensing means is hermetically secure, thus providing a long shelf-life.

10. Corners of pathways are rounded or curved so as to prevent build-up of substances at or around sharp corners. This also improves removal of articles from moulds during manufacture.

11. The wettability of surfaces of pathways can be improved by coating them with a fine layer of sugar or similar substance.

The invention has been described by way of example only and variations to it may be made without departing from the scope of the invention. For example, the apparatus may be formed from a material which is transparent to a band of radiation. An optical reader may be positioned adjacent analysis cells 16 so that a colour change in cell may be monitored or detected. The signal indicative of any colour change may be absorbed or reflected by the sample in the cell. Optical readers, such as an optical fibre, can be arranged to sample and detect colour variations, convert them into an electrical signal for display, analysis or transmittal to a remote laboratory for analysis. In the latter case transmittal may be, for example, via a telephone link or via a GSM connection. A system comprising the disc member, a reader and means for converting signals obtained by the reader into electronic signals, may be connected to a high speed electronic data link such as a modem. Thus configured the system could be used to transmit signals to a remote laboratory for an export opinion of the analysis. Alternatively a remote analysis may be performed using a computer, programmed to receive signals from the analysis cells. The computer thus programmed may be capable of performing hundreds of analysis or diagnosis.

CLAIMS

1. Apparatus for chemical analysis comprising: a member having formed therein a reaction chamber, which is in fluid communication with at least one analysis cell, at least one reservoir and at least one centrifuge chamber, the at least one centrifuge chamber is adapted to receive a sample, via an inlet port, so that, in use, the sample passes from the centrifuge chamber into the reaction chamber when the member is placed in a first orientation; the sample flows from the reaction chamber into a first reservoir, when the member is placed in a second orientation; the sample flows from the reservoir to the reaction chamber when the member is placed in a third orientation; and the sample flows from the reaction cell to at least one analysis cell when the member is placed in a fourth orientation.

2. Apparatus according to claim 1 wherein the first and third orientations are substantially identical, thereby causing or permitting the sample to return to a chamber or reservoir previously entered.

3. Apparatus according to claim 1 or 2 wherein the member is rotatable, in opposite senses, at an angular velocity which is less than the angular velocity of a centrifuge which supports the member.

4. Apparatus for chemical analysis comprising: a rotatable member having formed therein a sample inlet port communicating with a centrifuge chamber, a reaction chamber communicating with the centrifuge chamber by a fluid pathway, and at least one analysis cell communicating with the reaction chamber by a fluid pathway, wherein the rotatable member is spun, in use, in order to centrifuge a sample to be analysed within the centrifuge chamber, and wherein the rotatable member may subsequently be stepped for rotation at least partly in a vertical plane through fixed and predetermined arcs of circles so that the sample for analysis flows into the reaction chamber under the influence of gravity for reaction with one or more reagents, and the products of the reaction chamber subsequently flow into the at least one analysis cell.

5. Apparatus according to claim 2 or 3 wherein the rotatable member is preferably in the form of a flat disc..

6. Apparatus according to any preceding claim wherein the member comprises a synthetic plastics material having at least one surface which is substantially transparent to radiation in the visible waveband.

7. Apparatus according to any preceding claim wherein a plurality of beads or particles are provided, in use, in the reaction chamber, the said beads or particles having a reagent disposed on their surface.

8. Apparatus for chemical analysis comprising a rotatable member having formed therein a reaction chamber including an aggregate of beads for receiving on their surface a reagent, and at least one analysis cell communicating with the reaction chamber by a fluid pathway, wherein the rotatable member can be stepped for rotation through fixed and predetermined arcs of circle so that the products of the reaction chamber subsequently flow into the at least one analysis cell.

9. Apparatus according to any preceding claim wherein a fluid storage means is provided so that on compression of the fluid storage means, fluid is released into one or more chambers.

10. Apparatus for chemical analysis comprising: a rotatable member having formed therein a reaction chamber, at least one analysis cell communicating with the reaction chamber by a fluid pathway and storage means for introducing into the reaction chamber one or more liquids for acting on a sample, the storage means comprising a compressible, open-cell member adapted to be mounted within a chamber, the storage means, in use, contains a liquid, so that on compression the liquid is released into a fluid flow path communicating with the reaction chamber.

11. Apparatus according to claim 7 or any claim dependent thereon, wherein the beads are spherical and their average diameter is between 0.1 mm and 3 mm.

12. Apparatus according to claim 11 wherein the average diameter of the beads is between 0.5 and 0.7 mm.

13. Apparatus according to claim 9, wherein the fluid storage means is elongate in form, and has two opposite ends sealed.

14. Apparatus according to claim 1 wherein the member is in the form of a polygon having substantially flat edges.

15. Apparatus according to claim 1, 4, 8 or 10, including a plate cap member bonded to an upper surface of the member to close the chambers and pathways.

16. Apparatus according to any preceding claim, including an array of analysis cells, each cell having a relatively wide-bore inlet path communicating with said reaction chamber and a relatively narrow-bore outlet path communicating with a waste reservoir.

17. An adaptor plate for mounting the apparatus defined in any of Claims 1 to 16 to a rotor of a centrifuge.

18. A centrifuge including the adaptor of claim 17 and the apparatus defined in any of claims 1 to 16, the centrifuge being capable of being controlled so as to be able to operate selectively in a high speed centrifuge mode and a relatively low speed stepping mode in opposite senses, thereby enabling relatively slow clockwise and counter clockwise rotation of the member.

19. A method of chemical analysis comprising the steps of: providing a rotatable member mounted for rotation having at least a component in a vertical plane, the rotatable member having formed therein a centrifuge chamber, a reaction chamber communicating with the centrifuge chamber by a fluid pathway, and at least one analysis cell communicating with the reaction chamber by a fluid pathway,

introducing a sample for analysis into the centrifuge chamber and spinning the rotatable member to effect a centrifuge operation;

stopping the spinning operation and rotating the member through a predetermined arc of circle so that the centrifuge products flow, via a fluid flow path, under the influence of gravity to the reaction chamber,

treating the centrifuge products in the reaction chamber with one or more reagents, and

rotating the rotating member through a further predetermined arc of circle in order to permit the contents of the reaction chamber to flow under the influence of gravity into the at least one analysis cell.

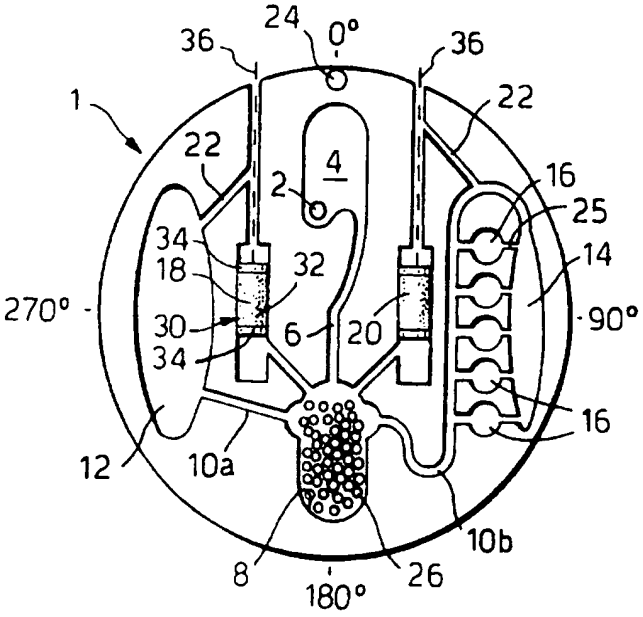
20. A method according to claim 19 including the steps of obtaining a signal from the at least one analysis cell and transmitting the signal to a remote location using a telecommunication link.

21. A method of diagnosis according to claim 19 or 20.

22. Apparatus substantially as herein described with reference to the Figures.

23. A method substantially as herein described with reference to the Figures.

Fig.1.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36
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22
22
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2
16
25
34
18
32
14
270°
90°
30
6
20
34
16
12
10a
10b
8
26
180°

Fig.5.

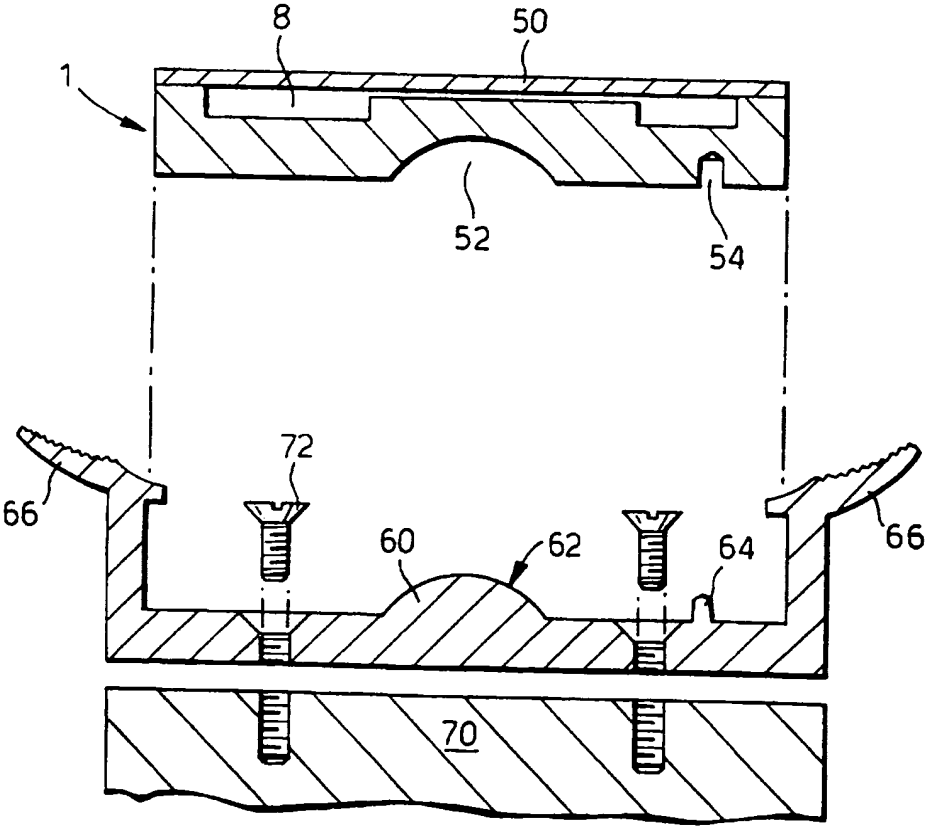
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Fig.2a.

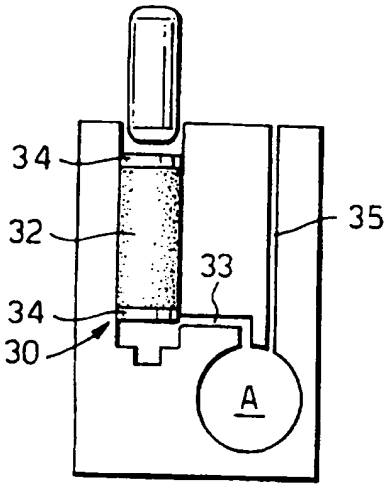


Fig.2b.

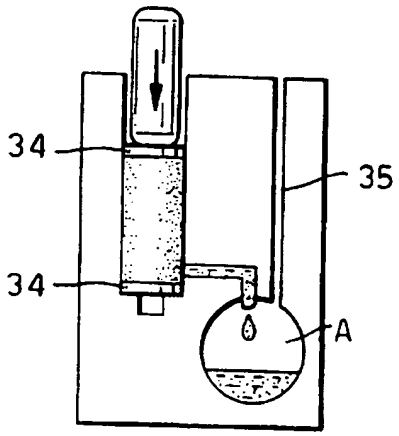


Fig.2c.

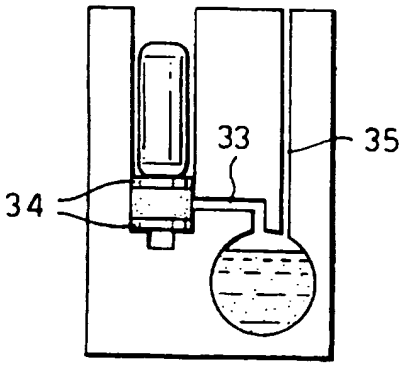


Fig.2d.

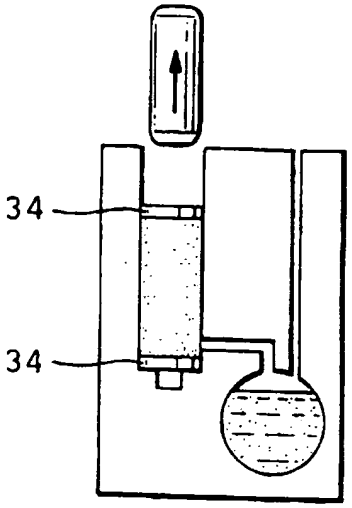


Fig.3a.

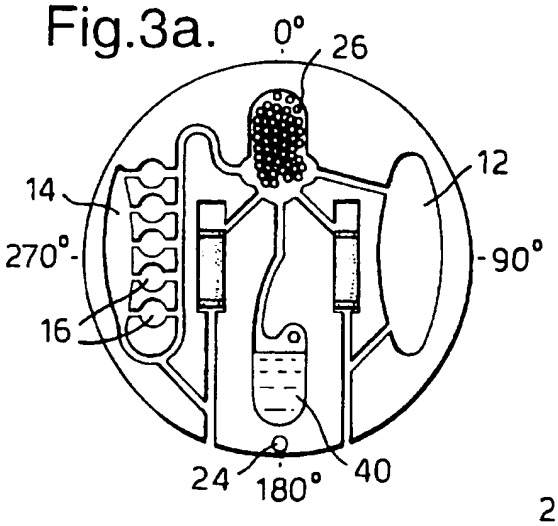


Fig.3b.

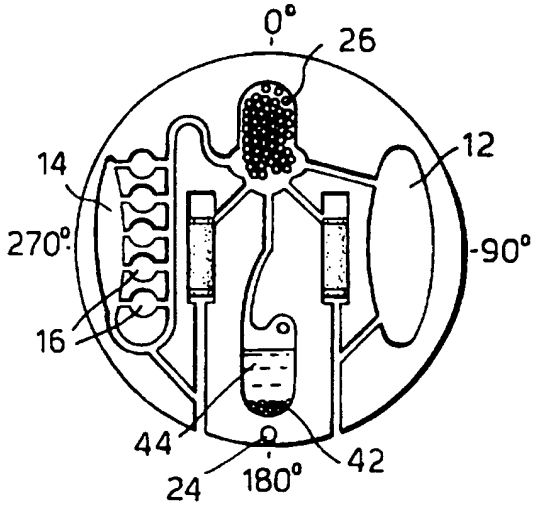


Fig.3c.

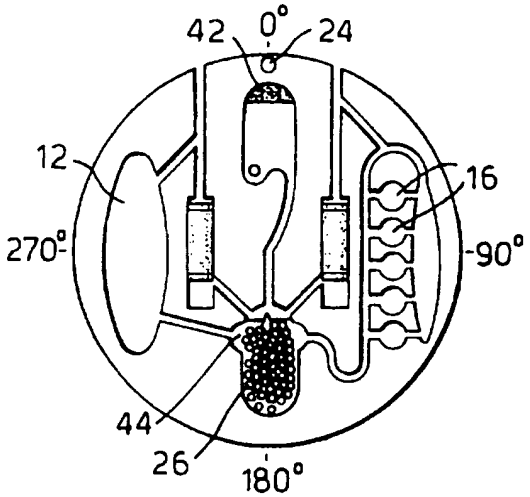


Fig.3d.

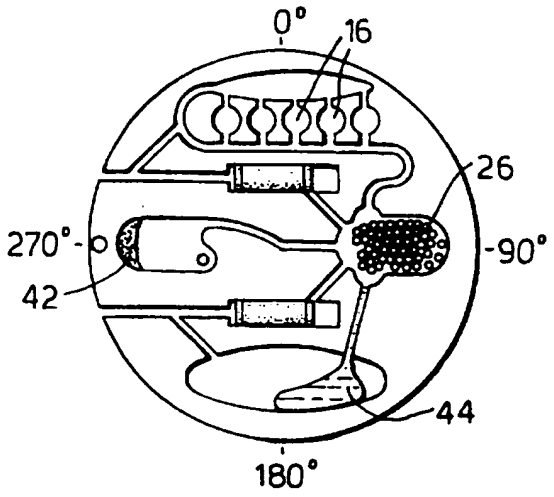


Fig.4a.

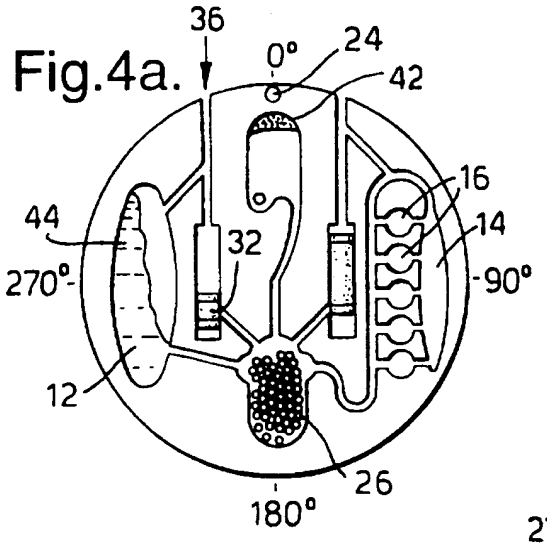


Fig.4b.

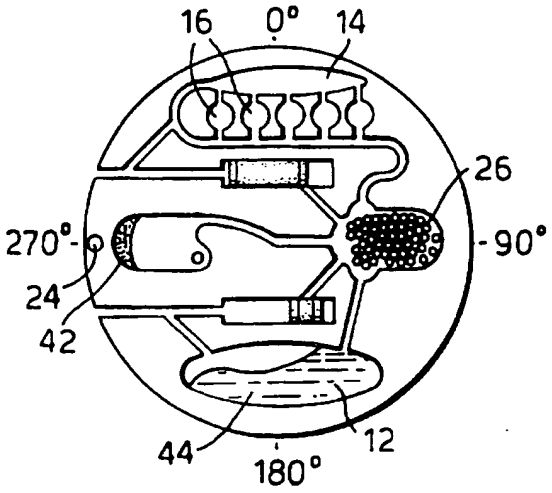


Fig.4c.

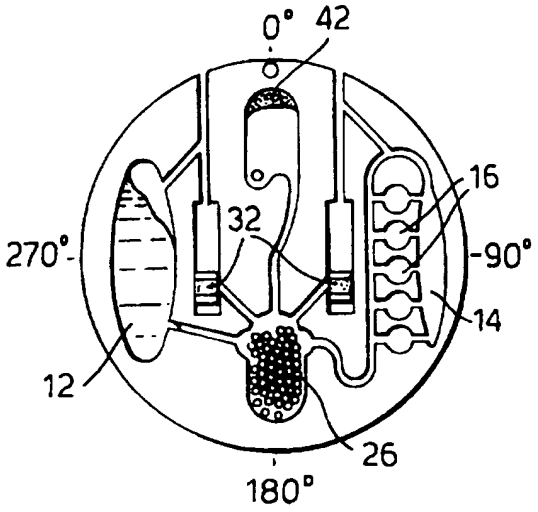
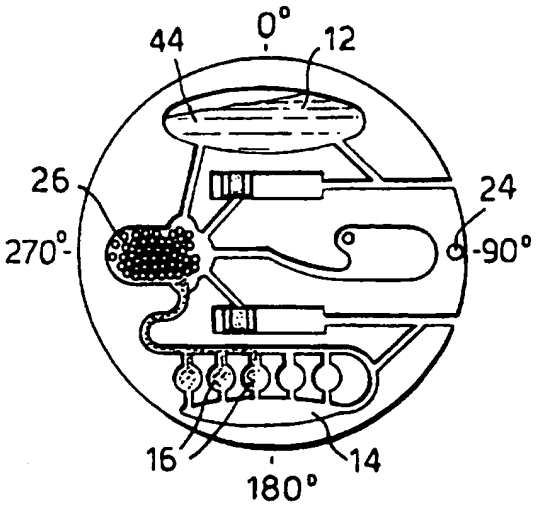


Fig.4d.



INTERNATIONAL SEARCH REPORT

Inter. ..onal Application No
PCT/GB 97/02661

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/07 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 297 394 A (MILES INC) 4 January 1989	1,3,6, 14-16
A	see column 10, line 37 - column 14, line 49; figures 3-7	4,8-10, 19
A	EP 0 197 865 A (GUIGAN JEAN) 15 October 1986 see page 6, line 18 - page 8, line 16; figures 3-20	1-10,15, 16,19
A	US 4 985 204 A (KLOSE SIGMAR ET AL) 15 January 1991 see figure 2; example 1	1-4,6,8, 14-16,19
A	US 5 160 702 A (KOPF-SILL ANNE R ET AL) 3 November 1992 cited in the application see column 7, line 4 - column 8, line 19	1,4,5,8, 10,19

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
29 January 1998	09.02.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hodson, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/GB 97/02661

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 162 237 A (MESSENGER LOWRY J ET AL) 10 November 1992 cited in the application see column 6, line 47 - column 7, line 2 see column 10, line 48 - column 12, line 24; figures 3-5 ---	1,2,4,6, 8-10,14, 15,19
A	EP 0 228 120 A (INSTRUMENTATION LAB SPA) 8 July 1987 see the whole document -----	17,18

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/02661

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 21
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body

2. [X] Claims Nos.: 22,23
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Claims 22 and 23 contain no features other than references to the description and the drawings. Their scope is therefore so unclear as to exclude a meaningful search.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. Jional Application No
PCT/GB 97/02661

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ional Application No
PCT/GB 97/02661

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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